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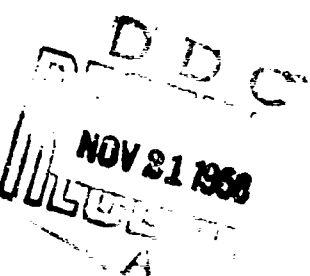
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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



ON A TUMOR CAUSED BY YEAST CELLS IN THE NASAL
CAVITY AND ITS SIDE CAVITIES OF THE HORSE

Zeitschrift fuer Veterinaerkunde
(Journal of Veterinary Sciences),
Berlin, Vol 47, 1935, pp. 111-116.

Dr. Schellner, Chief Staff Veterinarian at the Office for Army Veterinary Examination (Heeresveterinaeruntersuchungsamt), Berlin.

The literature dealing with yeasts grown from animal and especially human specimens is so extensive that the listing of the individual authors is impossible. In the field of veterinary medicine, it was Rivolta who, in 1872, discovered Cryptococcus farciminosus, the causative agent of Lymphangitis epizootica, a gemmiparous fungus, and thus led the attention to these yeast-like microorganisms. In the field of human medicine, the reports published by Busse and Buschke in 1894, still considered to be of fundamental significance, formed the start for the research on Blastomycetes as disease-causing agents in humans.

In addition to the Blastomycetes encountered in the horse by Rivolta [1] and Tokishiga [1] -- considered to be pathogenic, -- Ostertag described a specific muscle inflammation in cattle that is characterized by lentil-to-walnut sized, brittle, fibroma-like knotty muscular foci. Since yeast cells were identified in these muscular foci also, they were called generally Blastomycomas, although there are as yet no experimental transfer results available with positive findings.

The fact that many yeast-caused diseases in humans and in animals are characterized by tumor-like new formations, and the fact that proof is available on the basis of numerous reports showing that the various yeasts encountered are capable of generating these 'tumor-like' formations, played an important role in the etiology of malignant tumors for a long time. It is, nonetheless, a fact even today that no researcher has been able so far to create carcinoma from yeast strains originating from carcinomas, nor to create sarcoma from yeast strains originating from sarcomas. The investigations of Israel [2] and others led to the conclusion that yeast cells

entering the animal body by vaccination either perish or multiply vigorously and cause irritation as a foreign substance. This factor subsequently leads to more or less pronounced tissue production, to the formation of granulation tumors called Blastomycomas.

One would think that granulation tumors of this nature are not very rare in animals -- Lydia Rabinowitch [3] succeeded in finding among 50 yeasts grown from cow dung and juicy fruits and tested for pathogenicity, seven strains that caused pathological changes in mice and rabbits. On the other hand, there is the fact that there are only sporadic reports in the veterinary-medical literature -- apart from Lymphangitis epizootica in the horse and the above-mentioned Blastomycosis in cattle and hogs -- of yeast cells causing tumor-like growths. Since there has been only one single report in the literature dealing with the pathological anatomy of the horse [4] that describes a yeast-caused tumor formation in the nasal cavity of this animal (Gasse), we deem the publication of the following case and of the subsequent investigations as justified.

A serum-like, pussy secrete, taken from the mucous membrane of the right-side nostril, of yellowish color, of a horse treated for an empyema in the right-upper jaw, was submitted to H. V. U. A. [Office for Army Veterinary Examination] in March 1934. This horse was called 'Angriff.' The sample was forwarded for bacterioscopic and bacteriological examination. An examination of the puss in the fresh state showed the presence of numerous spherical and oval elements that varied considerably in size. Grain-like nuclei were visible in the interior of these elements. The cells showed clearly a double-contoured membrane; some, especially the larger ones, exhibiting a colorless surrounding around the cell. The refractive behavior of the yeast cells as contrasted with stainings with Loeffler's methylene blue according to Gram and Ziehl-Neelsen, was noted when the secrete swabbing was stained. Best results were achieved by staining according to Giemsa: although the yeast cells stained only weakly in this case also, their details were nonetheless clearly visible.

In addition to these yeast cells, no special microorganisms other than leucocytes, lymphocytes, and associated bacteria, were identifiable. It was therefore concluded that there is a suspicion of yeast cell-caused mucous membrane disease, justifying trepanation.

The histological examination of a portion of the tissue from the mucous membrane in the right-upper jaw cavity showed numerous sarcoma like cells that were located within a structure of connective tissue support, that were again infiltrated with yeast cells. The radical surgery was recommended on the basis of this finding. The operation could not be performed, however, since subsequent trepanation indicated that the tumor in the right-upper jaw cavity was so extensive that any operation could not have ensured success. The horse was declared as incurably sick. The autopsy results at H. V. U. A. justified the destruction of the animal. Except in the skull, no pathological-anatomical changes, including metastases caused

by yeast cells, were encountered.

The right side of the face was quite swollen in the nasal and upper jaw cavities, in comparison to the left side. About one finger width above right-side jawbone and three finger widths behind the nasal end of the jaw line, there was a trepanation hole of the size of a ten-Pfennig coin. The visible mucous membranes of the right-side nasal cavity were covered with crusts and a puss-like yellow-green fluid. After cleaving the head at the left side of the median line and removal of the nasal separation membrane, one could see a fist-sized tumor at the medial surface of the right-lower nose muscle, situated on the mucous membrane. Its shape and size caused a strong pressure on the adjoining portion of the upper nasal muscle. This formation extended cranially into the cranial cavity in the front: on the mucous membrane of this, there were eight spherical, cherry-stone to walnut sized growths. The same formations were evident also on the mucous membrane of the lateral wall of the right-side upper-jaw cavity and the jawbone cavity. On the latter, there was a walnut-size tumor. The surface of the yellowish tumorous mass is partially softened and changed into a non-odorous, yellow-green, tough, slimy fluid. This is evident also in the hanging sections of the jaw and nasal cavity. The consistency of the formation was soft, pudding-like; the surface of the cut, smooth, bacon-like, and shiny. The upper jawbone and the right side of the greatly enlarged upper jaw cavity were softened to an extent of 8 x 2 cm. Upon histological examination, the sections taken from various portions of the tumor stained with Hematoxylin-Eosine according to Gieson had the appearance of an unspecific granulation tissue. It consists of a slightly developed base tissue, a lattice-like tissue consisting of new connective tissue, fibroblasts, and small capillary vessels. There are mainly polymorphic-nucleus leucocytes and single-nucleus round cells; also some cells of variegated shape with one nucleus visible. The individual elements of this base tissue are quite well defined. Degeneration phenomena such as nucleus decomposition, are only very seldom observed. Round, pink formations, that appear the same as those encountered in the nasal secrete, are visible in all sections of the specimen when stained with Hematoxylin-Eosine. These formations vary in size; some are no larger than a red blood particle while others are twice or three times as large. Most are spherical; some, however, are oblong. Sprout formation was observed only rarely; wherever it was observed, it had the configuration typical of yeasts, exhibiting a strung appearance (see Fig. 3). Thus, the histological investigation showed a typical granulation tumor caused by yeasts.

The culture tests

The growing of the pure fungi could be accomplished both from the tumor tissue and from the nasal secrete; the latter was pretreated before processing for 10 minutes with a 1% antiformin solution. The growing was conducted on Nervina malt media (5 g peptone, 60 g Nervina malt, 5 g glycerol, 5 g NaCl, 18 g agar, and 1000 g water) at 22°C. As early as after 48 hour, there developed succulent round colonies of greyish white color

and moist greasy appearance, that could be readily grown in various generations. After 10 days, the color of the colonies became more yellowish; approximately 20 days later it became bright yellow. In order to determine the fermentative effect on carbohydrates, the following sugar media were inoculated:

Monosaccharides: a) pentoses arabinose and xylose; b) hexoses dextrose, levulose, mannose, and galactose -- a trisaccharide, raffinose; and the alcohols sorbitol, mannitol, dulcitol; and the polysaccharides dextrin, starch, and glycogen. The results were consistently negative after breeding for eight weeks; a slight fermentation of dextrose was evident after observation for several days.

The individual yeast cells showed in the suspended drop in the fresh state, a thin membrane that subsequently became thicker and exhibited a double contour. The inner protoplasm showed nucleus-like formations; sprouts were frequently visible but the formation of mycelles and spores was never observed. Accordingly, the culture behavior and the morphological picture indicated that the microorganisms encountered are Blastomycetes (Ascomycetes according to Buschke).

Animal tests

The inoculations were performed with culture suspensions. Mice, guinea pigs, rabbits, and two horses served as the experimental animals.

Three mice obtained 0.2 cu cm culture suspension orally; they died 14, 17, and 23 days thereafter, respectively. Three additional mice received 0.1 cu cm subcutaneously; they died 3, 6, and 11 days thereafter, respectively. Finally, three mice received 0.1 cu cm in tenfold dilution; they died after 3 and 7 days, respectively. Pure-culture yeasts could be grown from the heart blood of all animals; furthermore, there were sporadic yeast cells evident in the histological examination of the lungs, liver, and kidneys. Reactive inflammation of the tissue could not be observed. The guinea pigs that received 2.0 cu cm culture suspension orally showed one death after 10 days; here, too, the findings were the same as in the case of mice. Whereas guinea pigs that received 0.5 cu cm inoculating material subcutaneously remained healthy, from among three additional guinea pigs that were inoculated with 0.5 cu cm, two died after 60 and 73 days, respectively. Autopsy showed identical findings: enlargement of the body lymph nodes; blood coagulation in the large parenchyma, and specific uniform changes on the epiploon. This consisted of numerous millet-to-pea sized nodules that had the consistency and color of lymph nodes: they were identified as blastomycomas by histological and culture tests. Three rabbits that were administered 0.5 cu cm intravenously, 1.0 cu cm i. p., and 1.0 cu cm subcutaneously, respectively, remained healthy during the ten-month observation period.

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Fig. 1. Tumor (a). In tumor a in the cranial cavity; caused by yeast cells.

Section through the tumor shown in Fig. 1, at a yeast cells:

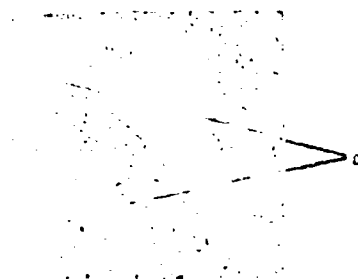


Fig. 2. Low magnification.

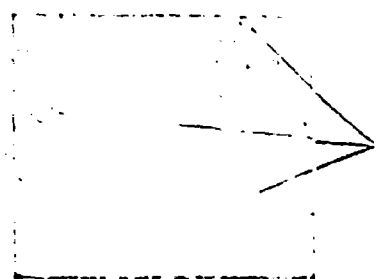


Fig. 3. High magnification.

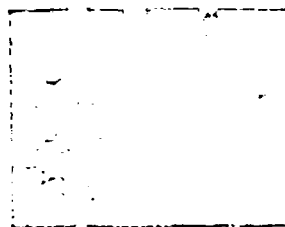


Fig. 4. Pure yeast culture on a maltose plate.

One of the horses received 1 cu cm culture suspension intravenously; the other was infected through the nose with a plug of cotton wool impregnated with concentrated culture suspension, inserted into both nostrils. The horses remained healthy; autopsy 10 months after the tests started showed that neither the rabbits nor the horses had blastomycotic formations in the area surrounding the vaccination site.

The results of the infection tests agreed with findings reported by other authors.

Summary

1. A report was presented on granulation growths located in the nasal, jaw, and cranial cavities of a horse, caused by yeast cells. These growths were characterized by progressive growth in all age states.

2. The active agent grown from the nasal secrete and from the tumor-like tissue proved to be a Blastomycete.

3. Vaccination tests on mice, guinea pigs, rabbits, and horses had the positive result of making it possible to cause tumor-like formations in the tissue of two guinea pigs which proved to be Blastomycetes on the basis of culture and histological examination.

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